Comparative Subchronic Toxicity Studies of Three Disinfectants

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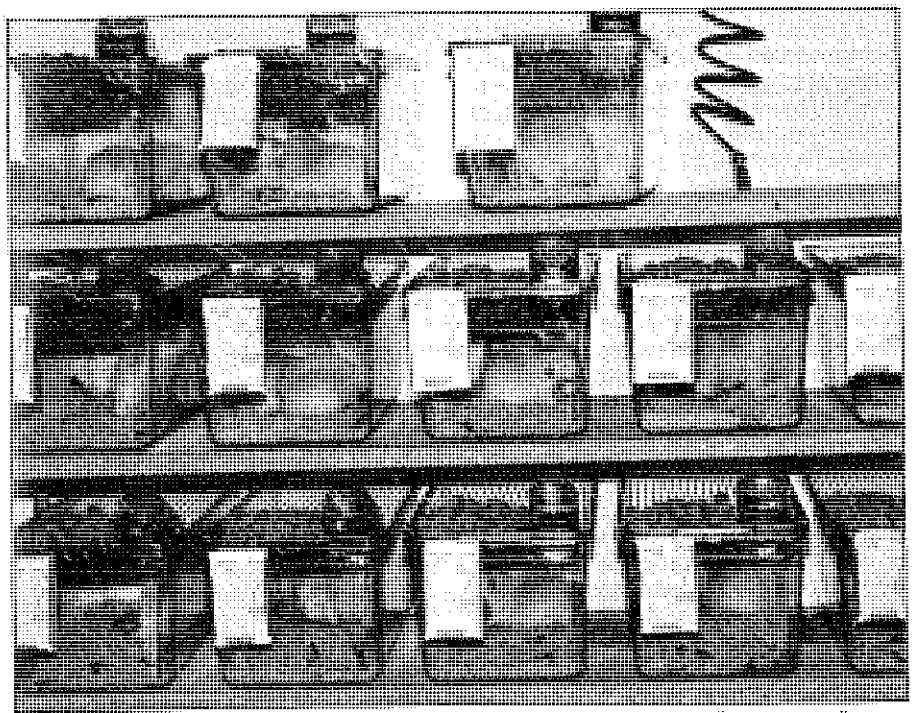
In separate subchronic toxicity studies, male and female Sprague–Dawley rats received various dosages of chlorine, monochloramine, or chlorine dioxide in their drinking water for 90 consecutive days. None of the disinfectants caused premature death at any of the concentrations used. The highest dose of chlorine tested (250 mg/L) was concluded to be a no observable adverse effect level. At 200 mg/L (the lowest observable adverse effect level), monochloramine produced decreased body and organ weights in both sexes and a small decrease in red blood cell count and serum calcium in males. Chlorine dioxide produced dosage-related decreases in body and organ weight at concentrations as low as 25 mg/L, but its most significant toxic effect was the induction, at all concentrations, of nasal lesions.

The principal goal of disinfecting water supplies is the elimination of pathogens that are responsible for waterborne diseases. Chlorination is a successful method for achieving this goal and has been the predominant method of drinking-water disinfection in the United States for more than 70 years. Chlorine is inexpensive and relatively convenient to produce, store, transport, and use. The toxicity of aqueous chlorine (technically a mixture of HOCl and OCl^-, depending on pH) has not been extensively studied, but its common usage puts it into the category generally regarded as safe.[1] Nonetheless, there has been concern in recent years about the health effects of trihalomethanes (THMs) and other by-products formed during chlorination when chlorine reacts with organic materials (including natural products) contained in surface water.[2]



Three hundred Sprague-Dawley rats were divided into experimental groups and were housed two per cage for various chemical treatments.

In 1980, the US Environmental Protection Agency (USEPA) evaluated alternate methods of drinking water disinfection that would produce lower levels of THMs. Considering toxicological as well as technological criteria, the Safe Drinking Water Committee judged three chemicals, i.e., chlorine dioxide, chloramine, and ozone, to be potentially suitable substitutes for chlorine as primary or secondary drinking water disinfection agents.[3] This article compares the oral toxicity of two of these alternate disinfectants (monochloramine and chlorine dioxide) with that of chlorine.

Chlorine dioxide (ClO_2) is used mainly as an industrial bleaching agent for wood pulp, textiles, flour, fats, oils, and waxes and has also been widely used at drinking water treatment plants for taste, odor, and algal control; iron and manganese removal; and (mainly in Europe) water disinfection.[4] Because it is unstable, sensitive to temperature, pressure, and light, and explosive in air at concentrations of about 4 percent or more, ClO_2 is usually generated and used on-site to avoid problems of bulk storage and distribution. Chlorine dioxide and its associated products, chlorate (ClO_3^-) and chlorite (ClO_2^-), have been studied in recent years and have toxicological properties that are of concern. In vivo, both compounds induce the production of hydrogen peroxide, which causes hemolytic anemia by oxidizing hemoglobin to the nonfunctional pigment methemoglobin.[5,6]

Chloramination is a technique that is being adopted by many communities as an alternative to the use of free chlorine for drinking-water disinfection. This process employs a combination of chlorine and ammonia treatment that produces predominantly monochloramine (NH_2Cl), which is much less reactive with the residual organics in water, producing lower levels of THMs and other halogenated by-products.[7] Al-

TABLE 1

Selected data on body weight, organ weight, water consumption, and daily dosage for rats exposed to chlorine in drinking water for 90 days

Parameter	Mean ± Standard Deviation				
	0 mg Cl/L	25 mg Cl/L	100 mg Cl/L	175 mg Cl/L	250 mg Cl/L
Males					
Initial body weight—*g*	322.4 ± 12.1	325.9 ± 12.6	329.9 ± 6.9	320.1 ± 7.4	332.1 ± 11.4
Final body weight—*g*	549.7 ± 50.7	527.6 ± 36.2	549.5 ± 47.0	511.0 ± 39.9	520.3 ± 25.8
Weight gain—*g*	227.3 ± 46.2	201.6 ± 35.1	219.7 ± 46.0	190.9 ± 37.9	188.2 ± 23.5
Organ weight—*g*					
Brain	2.10 ± 0.12	2.10 ± 0.10	2.13 ± 0.13	2.12 ± 0.10	2.11 ± 0.13
Testes	3.25 ± 0.56	3.58 ± 0.18	3.58 ± 0.35	3.26 ± 0.35	3.49 ± 0.30
Heart	1.58 ± 0.17	1.62 ± 0.18	1.55 ± 0.20	1.62 ± 0.14	1.60 ± 0.17
Kidneys	3.72 ± 0.34	3.64 ± 0.42	4.12 ± 0.42	3.86 ± 0.54	3.85 ± 0.32
Adrenals	0.10 ± 0.02	0.09 ± 0.02	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.03
Liver	15.82 ± 2.70	15.08 ± 1.51	15.71 ± 2.18	14.15 ± 1.15	14.47 ± 1.17
Lung	2.18 ± 0.16	2.06 ± 0.26	2.15 ± 0.26	1.99 ± 0.23	1.97 ± 0.17
Spleen	0.82 ± 0.11	0.85 ± 0.14	0.86 ± 0.12	0.77 ± 0.15	0.79 ± 0.08
Thymus	0.42 ± 0.07	0.41 ± 0.11	0.32 ± 0.08*	0.35 ± 0.04	0.35 ± 0.07
Water consumption—*mL/rat/d*†	54.4 ± 4.0	44.0 ± 1.5*	41.0 ± 3.4*	37.4 ± 3.0*	34.6 ± 2.9*
Calculated dose—*mg/kg/d*		2.1 ± 0.1	7.5 ± 0.7	12.8 ± 0.8	16.7 ± 1.9
Females					
Initial body weight—*g*	232.4 ± 6.8	233.4 ± 9.0	233.8 ± 9.2	225.9 ± 10.3	231.9 ± 11.4
Final body weight—*g*	309.0 ± 28.3	308.4 ± 29.4	307.9 ± 24.8	927.4 ± 24.7	301.8 ± 21.8
Weight gain—*g*	76.6 ± 24.7	75.0 ± 25.3	74.1 ± 19.5	71.5 ± 25.7	69.9 ± 14.2
Organ weight—*g*					
Brain	2.05 ± 0.13	1.98 ± 0.08	1.97 ± 0.10	1.99 ± 0.10	1.98 ± 0.11
Ovaries	0.21 ± 0.06	0.22 ± 0.04	0.21 ± 0.06	0.23 ± 0.08	0.21 ± 0.04
Heart	1.13 ± 0.10	1.09 ± 0.13	1.07 ± 0.13	1.05 ± 0.13	1.04 ± 0.11
Kidneys	2.25 ± 0.18	2.39 ± 0.33	2.31 ± 0.30	2.35 ± 0.21	2.47 ± 0.25
Adrenals	0.09 ± 0.03	0.12 ± 0.03	0.13 ± 0.03*	0.10 ± 0.02	0.10 ± 0.02
Liver	9.11 ± 1.01	8.99 ± 1.54	8.60 ± 0.93	8.92 ± 0.98	9.00 ± 1.57
Lung	1.52 ± 0.15	1.55 ± 0.18	1.58 ± 0.17	1.62 ± 0.19	1.51 ± 0.21
Spleen	0.68 ± 0.12	0.71 ± 0.07	0.65 ± 0.12	0.65 ± 0.12	0.69 ± 0.14
Thymus	0.41 ± 0.15	0.37 ± 0.10	0.38 ± 0.08	0.37 ± 0.05	0.38 ± 0.15
Water consumption—*mL/rat/d*†	48.1 ± 3.6	43.1 ± 2.5	38.7 ± 5.5*	33.1 ± 3.8*	29.9 ± 2.9*
Calculated dose—*mg/kg/d*		3.5 ± 0.4	12.6 ± 2.1	19.5 ± 2.0	4.9 ± 2.9

*Significantly different from control group; $p \leq 0.05$, Tukey's multiple-comparison procedure used
†$N = 5$, calculated on a per-cage basis and corrected for two rats per cage

though NH_2Cl is reported to be a less effective biocide than free chlorine, it forms a more stable residual in the distribution system and it reduces the unpleasant tastes and odors caused by the chlorination of aromatic compounds that are often present in chlorine-treated water.[8] Although the use of monochloramine in drinking-water disinfection has been associated with the production of methemoglobin in dialysis patients,[9] experimental studies have not confirmed this effect in laboratory animals.[10-13] Another health concern is evidence suggesting NH_2Cl may be genotoxic, producing mutations in bacteria[14] and abnormal mitotic figures in the livers of mice.[15]

The toxicity studies described here were conducted in order to provide direct comparative data on the subchronic effects of these disinfectants. Such studies will be useful in making rational decisions about the relative risks and benefits of chlorine and alternate oxidants for drinking water disinfection. Ozone was not considered in these studies because it (i.e., the oxidant) does not remain in water presented to the consumer.[16]

Methods

Test chemicals. The chlorine (CAS 7782-50-5) solution was prepared by bubbling chlorine gas* into double-distilled water to pH 9.4. The concentration was determined according to the diethyl-*p*-phenylenediamine (DPD) method[17] and reported in terms of available chlorine.

The monochloramine (CAS 10599-90-3) solution was synthesized by the addition of stock chlorine solution and ammonium hydroxide to a bicarbonate buffer of pH 9, as described by Abdel-Rahman et al.[11] NH_2Cl was determined by the DPD method.[17]

The chlorine dioxide (CAS 10049-04-4) solution was synthesized by purging ClO_2 from an acidified sodium chlorite ($NaClO_2$) generator through an absorbent $NaClO_2$ solid column into distilled deionized water and was measured spectrophotometrically as described elsewhere by Bercz et al.[13]

Amber-colored glass drinking-water bottles were used for each disinfectant to reduce photolytic degradation. Double-balled stainless-steel sipper tubes were used to minimize drippage and to facilitate accurate water consumption analysis. The bottles were filled to the top with fresh drinking solutions every other day. The concentration and the purity of the disinfectant solutions were determined before offering the test chemical to the animals and at the time of refilling bottles to determine the extent of degradation. The percentage of decomposition for chlorine, NH_2Cl, and ClO_2 during 72 h in the water bottles was 3.6–17, 6.8–10.8, and 3.6–7.3 percent, respectively.

Animals. Viral antibody-free, Crl:CD BR, Sprague-Dawley rats† were used for each toxicity study. The animals were approximately 70 days of age when received, with males weighing 300–325 g and females weighing 225–250 g. The animals were housed two per cage by sex in hanging polycarbonate cages containing hardwood chip bedding‡ and were held in quarantine for 10 days. The rats were maintained in a temperature (20–22°C) and humidity (40–60 percent) controlled room on a 12:12-h light cycle. Commercial rodent food§ and drinking water were given ad libitum. All aspects of this study adhered to the standards and practices endorsed by the American Association for Accreditation of Laboratory Animal Care.

Toxicity studies. Three hundred animals were divided into experimental groups for various chemical treatments using a computer-generated set of random numbers. Animals were individually identified by ear tag, and groups were tracked via color-coded identification cards on the cages, indicating the animal and treatment group. Each experimental group, consisting of 10 males and 10 females, was treated for 90 consecutive days. There were four treatment levels of each disinfectant: chlorine—25, 100, 175, and 250 mg/L; NH_2Cl—25, 50, 100, and 200 mg/L; and ClO_2—25, 50, 100, and 200 mg/L. In each study, a control

*Wright Brothers, Cincinnati, Ohio
†Charles River Laboratories, Portage, Mich.
‡Absorb Dri, Maywood, N.Y.
§Rodent Chow 5002, Ralston-Purina Co., St. Louis, Mo.

TABLE 2

Selected hematological and clinical chemistry values for rats treated with chlorine in drinking water for 90 days

Parameter	Mean ± Standard Deviation				
	0 mg Cl/L	25 mg Cl/L	100 mg Cl/L	175 mg Cl/L	250 mg Cl/L
Males					
RBC × 10^6	7.91 ± 0.37	7.96 ± 0.39	7.55 ± 0.33	7.51 ± 0.68	7.87 ± 0.37
WBC × 10^3	7.56 ± 1.22	8.70 ± 1.66	8.11 ± 3.77	8.26 ± 2.07	7.83 ± 1.56
Hgb—*g/dL*	15.05 ± 0.52	15.33 ± 0.70	15.26 ± 0.51	14.96 ± 1.16	15.87 ± 0.55
Hct—*percent*	43.72 ± 2.23	43.71 ± 2.45	41.89 ± 2.43	41.65 ± 3.86	44.36 ± 2.33
MCV—u^3	55.10 ± 0.74	54.80 ± 1.23	55.50 ± 1.43	55.50 ± 1.18	56.40 ± 1.43
Glu—*mg/dL*	156.40 ± 20.21	146.60 ± 14.56	169.70 ± 19.57	172.80 ± 19.00	171.50 ± 25.75
BUN—*mg/dL*	19.09 ± 3.22	19.99 ± 2.31	20.21 ± 4.41	20.00 ± 3.88	19.02 ± 4.24
Creat—*mg/dL*	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01*	0.05 ± 0.01
PO_4—*mg/dL*	6.48 ± 0.82	6.83 ± 0.31	7.28 ± 0.96*	7.26 ± 0.57*	6.56 ± 0.45
AST—*U/L*	99.10 ± 14.42	109.70 ± 24.31	124.20 ± 93.35	111.50± 24.60	112.20 ± 20.76
ALT—*U/L*	40.90 ± 6.31	40.40 ± 8.14	59.90 ± 71.29	47.40 ± 17.41	34.80 ± 10.02
Chol—*mg/dL*	63.30 ± 12.10	62.40 ± 11.73	67.00 ± 15.41	67.30 ± 36.14	62.00 ± 12.40
LDH—*U/L*	711.5 ± 310.3	1,035.5 ± 429.1	545.5 ± 295.1	1,056.5 ± 414.7	1,181.0 ± 415.4
Ca—*mg/dL*	10.03 ± 0.76	10.10 ± 0.31	10.36 ± 0.44	10.22 ± 0.08	10.10 ± 0.36
Females					
RBC × 10^6	7.10 ± 0.27	6.95 ± 0.26	7.19 ± 0.31	7.06 ± 0.60	7.00 ± 0.76
WBC × 10^3	4.30 ± 1.37	5.33 ± 2.40	5.25 ± 1.43	5.21 ± 2.46	6.94 ± 4.13
Hgb—*g/dL*	13.92 ± 0.40	13.99 ± 0.53	14.59 ± 0.53*	14.39 ± 1.09†	14.10 ± 1.71
Hct—*percent*	40.74 ± 1.77	39.32 ± 1.53	40.91 ± 1.68	39.87 ± 3.15	39.66 ± 4.70
MCV—u^3	57.20 ± 1.40	56.30 ± 0.95	56.80 ± 0.79	56.30 ± 1.49	56.40 ± 1.90
Glu—*mg/dL*	142.20 ± 17.76	128.20 ± 14.83	132.20 ± 15.95	132.50 ± 24.87	141.10 ± 25.55
BUN—*mg/dL*	15.61 ± 2.49	16.08 ± 1.64	16.10 ± 1.98	17.25 ± 2.63	15.16 ± 3.14
Creat—*mg/dL*	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
PO_4—*mg/dL*	5.02 ± 0.58	6.05 ± 0.98	6.66 ± 0.90*	6.62 ± 0.70*	6.09 ± 0.96
AST—*U/L*	70.00 ± 8.46	124.10 ± 129.70	88.40 ± 11.75	122.10 ± 69.71*	87.80 ± 23.28
ALT—*U/L*	27.60 ± 8.64	52.10 ± 75.11	32.10 ± 9.10	40.60 ± 27.22	6.20 ± 6.68
Chol—*mg/dL*	70.00 ± 16.97	74.40 ± 28.70	69.60 ± 18.36	85.20 ± 21.80	58.90 ± 12.05
LDH—*U/L*	213.9 ± 52.4	468.7 ± 302.6	360.0 ± 166.2	485.0 ± 388.4	417.5 ± 334.8
Ca—*mg/dL*	10.31 ± 0.36	10.28 ± 0.71	10.46 ± 0.44	10.73 ± 0.61	10.38 ± 0.51

*Significantly different from control group; $p \leq 0.05$, multiple-comparison procedure based on Tukey's studentized range test unless otherwise indicated

group received distilled water buffered with sodium bicarbonate to a pH of 8.0–8.5 (for chlorine and NH_2Cl) and pH 4.7 (for ClO_2). Treatment of the females was started on one day and of the males the following day.

All rats were observed daily for physiological and behavioral responses and for mortality. All unscheduled deaths were necropsied to determine the cause of mortality. Body weights were determined initially, then weekly throughout the study, and at termination. Food and water consumption was measured by weighing the food and water bottles (weekly for food and three times a week for water) throughout the study. Animals were fasted for approximately 18 h before necropsy. Prior to necropsy, each animal was anesthetized (pentobarbital; 60 mg/kg, intraperitoneally), the chest cavity was opened, and two 5-mL samples of blood were taken via cardiac puncture—one for hematological analysis and one for serum clinical chemistries.

Hematology samples were evaluated* for white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hgb), hematocrit (Hct), and mean corpuscular volume (MCV). Differential leukocyte counts (Wright-Giemsa stain) were determined by classifying 100 white blood cells into the following categories: lymphocytes, monocytes, eosinophils, segmented neutrophils, and basophils.

Serum clinical chemistry levels were determined using diagnostic kits and a chemistry analyzer.† The following clinical chemistry determinations were performed on the serum: glucose (Glu), blood urea nitrogen (BUN), creatinine (Creat), inorganic phosphate (PO_4), serum aspartate transaminase (AST), serum alanine transaminase (ALT), cholesterol (Chol), lactate dehydrogenase (LDH), and calcium (Ca).

The brain, liver, spleen, lung with the lower half of the trachea, thymus, kidneys, adrenal glands, heart, and gonads of each animal were weighed and grossly examined at necropsy. In addition to these tissues, the skin, mandibular and mesenteric lymph nodes, mammary gland, thigh muscle, sciatic nerve, sternebrae, thymus, esophagus, stomach, duodenum, jejunum, tongue, salivary gland, ileum, colon, cecum, rectum, pancreas, urinary bladder, seminal vesicles, prostate, uterus, nasal cavity and turbinates, pituitary, preputial or clitoral gland, Zymbal's gland, aorta, thyroid, parathyroids, and any gross lesions were examined and preserved in 10 percent neutral buffered formalin. Subsequently, all the tissues from males and females that had received the high dosage and from half the males and females in the control group were trimmed, processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All prepared slides were subject to histopathological analysis by a veterinary pathologist. Inflammatory and degenerative lesions were graded from one to four (minimal, mild, moderate, or marked), depending on the severity of the lesions. If target organs could be identified, those tissues from the lower-dosage groups were trimmed, processed, stained, and examined.

Statistical evaluation. Males and females were considered separately in all statistical analyses. A one-factor (dosage) analysis of variance (ANOVA) was used to analyze normally distributed measures: body weights, organ weights, organ weight ratios, food and water consumption, hematology, and clinical chemistry.[18] When a treatment effect was noted ($\alpha = 0.05$, F-test), the difference between the control and treatment groups was examined using Tukey's multiple comparison procedure.[19] For those hematological and clinical chemistry measures that were not normally distributed (extreme values, high variability), a nonparametric rank procedure, the Kruskal–Wallis test, was used to determine differences among the groups.[20] If a significant difference was found ($\alpha = 0.05$), differences among treatment groups were tested using a multiple-comparison procedure based on the Kruskal–Wallis test.[20] Incidence of histopathological lesions was analyzed by a Fisher Exact test, with $\alpha = 0.05$ as the criterion of significance.[21] Results of

*Model ZBI Coulter Counter, Coulter Electronics, Hialeah, Fla.

†Baker, Allentown, Pa.

TABLE 3

Selected data on body weight, organ weight, water consumption, and daily dosage for rats exposed to monochloramine in drinking water for 90 days

Parameter	Mean ± Standard Deviation				
	0 mg NH_2Cl/L	25 mg Cl/L	50 mg NH_2Cl/L	100 mg NH_2Cl/L	200 mg NH_2Cl/L
Males					
Initial body weight—*g*	305.0 ± 10.1	305.0 ± 12.7	304.5 ± 7.8	313.0 ± 8.5	304.3 ± 5.6
Final body weight—*g*	554.3 ± 63.8	525.1 ± 37.1	502.3 ± 33.4	506.3 ± 39.6	435.0 ± 27.0*
Weight gain—*g*	249.3 ± 56.8	220.1 ± 27.7	197.8 ± 33.9*	193.2 ± 35.1*	130.7 ± 25.4*
Organ weight—*g*					
Brain	2.16 ± 0.11	2.10 ± 0.11	2.07 ± 0.11	2.10 ± 0.12	2.07 ± 0.11
Testes	3.49 ± 0.24	3.37 ± 0.32	3.49 ± 0.28	3.62 ± 0.37	3.37 ± 0.21
Heart	1.56 ± 0.22	1.53 ± 0.12	1.57 ± 0.20	1.56 ± 0.16	1.34 ± 0.09*
Kidneys	3.85 ± 0.48	3.89 ± 0.45	3.71 ± 0.35	3.80 ± 0.33	3.43 ± 0.33
Adrenals	0.08 ± 0.01	0.09 ± 0.03	0.08 ± 0.02	0.07 ± 0.03	0.06 ± 0.02
Liver	15.61 ± 3.09	14.36 ± 1.53	13.41 ± 1.57	13.08 ± 1.35*	11.18 ± 1.22*
Lung	2.23 ± 0.30	2.03 ± 0.24	1.93 ± 0.23*	2.04 ± 0.18	1.72 ± 0.16*
Spleen	0.82 ± 0.09	0.80 ± 0.14	0.76 ± 0.08	0.78 ± 0.09	0.62 ± 0.17*
Thymus	0.40 ± 0.08	0.43 ± 0.11	0.31 ± 0.06	0.39 ± 0.07	0.30 ± 0.07
Water consumption—*mL/rat/d*†	64.4 ± 16.0	37.8 ± 3.1*	34.1 ± 1.9*	29.1 ± 1.9*	19.7 ± 1.4*
Calculated dose—*mg/kg/d*		1.8 ± 0.2	3.4 ± 0.2	5.8 ± 0.6	9.0 ± 0.5
Females					
Initial body weight—*g*	227.0 ± 16.6	230.3 ± 12.5	232.0 ± 16.7	234.6 ± 7.3	233.1 ± 8.1
Final body weight—*g*	313.6 ± 11.1	308.9 ± 14.5	309.7 ± 21.9	310.3 ± 12.7	278.6 ± 15.4*
Weight gain—*g*	88.3 ± 17.4	77.2 ± 11.2	77.8 ± 24.7	75.6 ± 10.8	45.4 ± 14.5*
Organ weight—*g*					
Brain	2.00 ± 0.11	1.96 ± 0.13	1.95 ± 0.18	2.01 ± 0.10	1.90 ± 0.13
Ovaries	0.23 ± 0.04	0.23 ± 0.05	0.24 ± 0.09	0.21 ± 0.04	0.21 ± 0.05
Heart	1.15 ± 0.07	1.11 ± 0.10	1.09 ± 0.10	1.11 ± 0.12	1.00 ± 0.16
Kidneys	2.33 ± 0.20	2.25 ± 0.21	2.39 ± 0.22	2.40 ± 0.15	2.37 ± 0.18
Adrenals	0.13 ± 0.05	0.12 ± 0.04	0.14 ± 0.03	0.10 ± 0.02	0.11 ± 0.04
Liver	10.08 ± 1.63	8.82 ± 0.83	9.19 ± 1.12	9.14 ± 0.66	7.69 ± 0.79*
Lung	1.57 ± 0.14	1.60 ± 0.09	1.53 ± 0.12	1.52 ± 0.19	1.42 ± 0.18
Spleen	0.71 ± 0.12	0.64 ± 0.06	0.69 ± 0.08	0.67 ± 0.08	0.56 ± 0.07*
Thymus	0.41 ± 0.09	0.36 ± 0.10	0.41 ± 0.08	0.33 ± 0.06	0.29 ± 0.08*
Water consumption—*mL/rat/d*†	48.9 ± 4.0	31.4 ± 0.8*	26.4 ± 1.9*	23.9 ± 1.2*	16.8 ± 2.6*
Calculated dose—*mg/kg/d*		2.6 ± 0.1	4.3 ± 0.4	7.7 ± 0.0	12.1 ± 1.7

*Significantly different from control group; $p \leq 0.05$, multiple-comparison procedure based on Tukey's studentized range test if ANOVA was applied
†$N = 5$; calculated on a per cage basis and corrected for two rats per cage

TABLE 4

Selected hematological and clinical chemistry values for rats treated with monochloramine in drinking water for 90 days

Parameter	Mean ± Standard Deviation				
	0 mg NH_2Cl/L	25 mg NH_2Cl/L	50 mg NH_2Cl/L	100 mg NH_2Cl/L	200 mg NH_2Cl/L
Males					
RBC × 10^6	7.8 ± 0.3	7.5 ± 0.5	7.6 ± 0.2	7.4 ± 0.3	7.4 ± 0.2*
WBC × 10^3	7.6 ± 2.2	7.7 ± 2.2	7.5 ± 0.8	8.1 ± 1.7	6.6 ± 1.2
Hgb—*g/dL*	15.1 ± 0.5	14.9 ± 0.8	15.0 ± 0.5	14.8 ± 0.4	14.7 ± 0.5
Hct—*percent*	43.2 ± 2.0	41.5 ± 3.0	41.9 ± 1.8	40.3 ± 2.3*	40.8 ± 1.5
MCV—u^3	55.4 ± 1.4	54.9 ± 0.9	55.2 ± 1.5	54.5 ± 1.5	55.2 ± 1.7
Glu—*mg/dL*	158.5 ± 17.6	153.2 ± 19.7	139.6 ± 20.3	137.50 ± 25.2	139.4 ± 27.6
BUN—*mg/dL*	16.75 ± 0.93	15.48 ± 2.06	16.37 ± 2.20	15.75 ± 1.85	19.03 ± 4.25
Creat—*mg/dL*	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.06 ± 0.01
PO_4—*mg/dL*	7.36 ± 0.74	7.01 ± 0.58	7.18 ± 0.64	7.43 ± 0.62	7.36 ± 0.44
AST—*U/L*	90.50 ± 21.58	76.00 ± 19.62	80.00 ± 9.47	79.60 ± 14.16	83.33 ± 12.38
ALT—*U/L*	42.50 ± 11.45	37.10 ± 9.30	38.44 ± 8.60	32.20 ± 4.64	38.44 ± 7.89
Chol—*mg/dL*	60.30 ± 27.00	56.80 ± 9.52	50.44 ± 13.45	53.20 ± 9.53	55.56 ± 12.03
LDH—*U/L*	638.0 ± 345.9	549.5 ± 438.7	808.3 ± 245.7	496.5 ± 191.4	558.9 ± 215.7
Ca—*mg/dL*	11.44 ± 0.56	10.36 ± 0.46*	10.43 ± 0.46*	9.93 ± 0.40*	9.77 ± 0.61*
Females					
RBC × 10^6	7.2 ± 0.3	7.2 ± 0.3	7.1 ± 0.3	7.1 ± 0.2	7.0 ± 0.5
WBC × 10^3	7.7 ± 2.2	4.8 ± 1.7*	6.6 ± 1.5	5.4 ± 0.9	5.5 ± 2.2
Hgb—*g/dL*	14.2 ± 0.6	14.5 ± 0.5	14.4 ± 0.3	14.5 ± 0.6	14.4 ± 0.7
Hct—*percent*	40.6 ± 1.6	40.3 ± 1.7	40.9 ± 2.1	40.4 ± 1.7	39.4 ± 2.1
MCV—u^3	56.6 ± 2.2	56.1 ± 1.3	57.3 ± 1.7	57.0 ± 1.6	56.5 ± 1.4
Glu—*mg/dL*	148.8 ± 6.4	146.50 ± 10.2	140.8 ± 15.0	145.1 ± 15.2	140.5 ± 33.1
BUN—*mg/dL*	27.31 ± 6.27	20.23 ± 4.64	16.61 ± 1.82*	17.82 ± 2.13*	21.16 ± 5.90
Creat—*mg/dL*	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
PO_4—*mg/dL*	5.73 ± 0.72	6.19 ± 0.48	6.70 ± 0.47	5.44 ± 0.35	5.71 ± 0.72
AST—*U/L*	126.89 ± 71.83	104.50 ± 32.24	83.50 ± 18.49	84.20 ± 12.51	111.60 ± 17.44
ALT—*U/L*	60.33 ± 62.15	33.10 ± 12.94	31.10 ± 6.06	26.00 ± 5.93	37.80 ± 12.36
Chol—*mg/dL*	94.78 ± 34.34	74.90 ± 15.74	66.20 ± 17.86*	78.60 ± 8.49	70.30 ± 14.45
LDH—*U/L*	324.7 ± 111.0	562.7 ± 268.7	472.0 ± 321.3	436.0 ± 219.9	621.0 ± 323.4
Ca—*mg/dL*	10.40 ± 0.51	10.43 ± 0.29	10.53 ± 0.39	10.11 ± 0.29	11.38 ± 0.39*

*Significantly different from control group; $p \leq 0.05$, multiple-comparison procedure based on Tukey's studentized range test unless otherwise indicated

the white blood cell differentials and gross necropsy were not statistically analyzed.

Results

Chlorine. The concentrations of chlorine that were used for this study were selected after reviewing the results of a 10-day pilot study, which demonstrated that up to 250 mg/L was well tolerated by Sprague-Dawley rats (data not shown). Accordingly, there were no deaths attributed to 90 days of dosing with any concentration of chlorine; however, daily water consumption decreased in both sexes relative to the controls in a dose-related manner (apparently as a result of taste aversion), reaching statistical significance in females at 100, 175, and 250 mg/L and in males at all treatment levels (Table 1). When daily water consumption was normalized by individual body weight, the same trend and statistically significant findings were obtained (data not shown). Total food consumption for animals of both sexes treated with chlorine did not differ significantly from the controls. Average calculated daily doses for the 20-, 100-, 175-, and 250-mg/L groups were, respectively, 3.5, 12.6, 19.5, and 24.9 mg/kg/d for females and 2.1, 7.5, 12.8, and 16.7 mg/kg/d for males (Table 1).

Initial and final body weights, weight gain, and organ weights are presented in Table 1. There were no significant differences between treated and control animals in any of these measures, except for increased average thymus weight in males (0.42 ± 0.07 versus 0.32 ± 0.08 g) and increased average adrenal weight in females (0.13 ± 0.03 versus 0.09 ± 0.03 g) consuming 100 mg/L chlorine. Analysis of the organ-weight-to-body-weight ratios revealed only three significant differences: increased relative kidney weight at 250 mg/L (0.82 ± 0.08 versus 0.73 ± 0.07 g), increased relative adrenal weight at 100 mg/L (0.04 ± 0.01 versus 0.03 ± 0.01 g) in females, and decreased relative thymus weight (0.06 ± 0.01 versus 0.08 ± 0.01 g) at 100 mg/L in males. One high-dosage female developed splenomegaly (three times more massive than normal); the exclusion of this animal did not affect the statistical results.

The results of the whole blood analyses and clinical chemistry are summarized in Table 2. The average Hgb concentration was significantly increased in females at 100 and 175 mg/L chlorine. The PO_4 levels were significantly increased in both sexes at 100 and 175 mg/L, AST levels were increased in females at 175 mg/L, and Creat was significantly decreased in males at 175 mg/L. With one exception, the WBC differentials revealed no significant changes in neutrophil, lymphocyte, monocyte, eosinophil, or basophil percentages for either sex at any dosage of chlorine. In males, the monocyte fraction was significantly decreased (2.4 ± 1.0 versus 5.20 ± 2.4 percent) at 175 mg/L. These hemotological and clinical changes were sporadic and, thus, not considered treatment-related.

All gross and microscopic observations during the 90-day subchronic study were considered to represent common, spontaneous lesions typical for Sprague-Dawley rats and were judged not related to exposure to chlorine.

Monochloramine. The concentrations of NH_2Cl were selected on the basis of a 10-day pilot study. No deaths were attributable to 90 days of dosing with any concentration of monochloramine that was used in this study. However, as in the case of chlorine, the daily water consumption decreased significantly in both sexes at all concentrations in a dosage-related fashion (Table 3). Average daily food consumption was not significantly different in treated females relative to controls. Males, however, showed a dosage-related decrease in food consumption, which reached statistical significance at 200 mg/L (24.0 ± 2.3 versus 31.3 ± 2.6 g/d). Normalization of food intake by final body weight indicated that, with the exception of females at 25 mg/L, neither sex exhibited food consumption that was different from the controls (data not shown). Average daily dosages calculated from water consumption and body weight data for the 25, 50, 100, and 200 mg/L groups were, respectively, 2.6, 4.3, 7.7, and 12.1 mg/kg/d for the females and 1.8, 3.4, 5.8, and 9.0 mg/kg/d for the males (Table 3).

At 200 mg NH_2Cl/L, both sexes showed a significantly decreased final body weight relative to the controls (Table 3). Mean weight gain was also significantly reduced relative to the controls in females at 200 mg/L and in males at doses of 50 mg/L or greater (Table 3). The highest dose level (200 mg/L) produced significant decreases in the absolute weight of the spleen, liver, and thymus of females and of the spleen, liver, lung, and heart of males relative to the controls (Table 3). At 100 mg/L, males had significantly decreased liver weight and at 50 mg/L significantly decreased lung weight compared with the controls. The 25-mg/L dose level had no significant effect on absolute organ weights in either sex. Statistical analysis of the relative organ weights showed that females had significantly increased relative kidney (0.85 ± 0.06 versus 0.74 ± 0.06 g) and decreased relative liver (2.76 ± 0.25 versus 3.22 ± 0.52 g) weights in the 200-mg/L-dose group compared with the controls. Males in the high dosage group had significantly increased relative brain (0.48 ± 0.03 versus 0.39 ± 0.04 g), testes (0.78 ± 0.06 versus 0.64 ± 0.07 g), and kidney (0.79 ± 0.07 versus 0.70 ± 0.03 g) weights.

The results of the selected hematological parameters are summarized in Table 4. The WBC count was significantly reduced in females at 25 mg/L but not at any other concentration of NH_2Cl. Males had significantly decreased Hct at 100 mg/L and RBC counts at 100 and 200 mg/L (7.4 ± 0.3 and 7.4 ± 0.2 versus $7.8 \pm 0.3 \times 10^6$ cells/mL).

There were no differences in the WBC differentials for any concentration of NH_2Cl (Table 4). Table 4 also shows the results of the serum clinical chemistry analyses. In females, Ca levels were elevated at 200 mg/L, whereas in males they were significantly depressed at all NH_2Cl concentrations, compared with control values. In the females, BUN levels were significantly decreased at 50 and 100 mg/L but not at 200 mg/L, and Chol levels were significantly reduced in females only at 50 mg/L.

All gross and microscopic observations were considered to represent common, spontaneous lesions in Sprague-Dawley rats, and no findings were deemed related to the 90-day exposure to NH_2Cl in the drinking water.

Chlorine dioxide. There were no deaths attributable to 90 days of dosing with any concentration of ClO_2 employed, but the daily water consumption decreased in both sexes in a dosage-related fashion, achieving statistical significance at 25, 50, 100, and 200 mg/L in females, and 50, 100, and 200 mg/L in males (Table 5). When water usage was normalized by final body weight, the same dosage-related pattern and levels of statistical significance were observed (data not shown). Average daily food consumption of the males was significantly reduced at 200 mg/L (26.2 ± 1.0 versus 29.0 ± 1.7 g/d). Average daily doses calculated from water consumption and body weight data for the 25-, 50-, 100-, and 200-mg/L groups were, respectively, 2.4, 4.6, 8.2, and 14.9 mg/kg/d for females, and 1.9, 3.6, 6.2, and 11.5 mg/kg/d for males (Table 5).

Both sexes showed significantly decreased final body weights at 200 mg ClO_2/L, and the mean weight gain was also significantly reduced at 200 mg/L for both sexes (Table 5). Mean absolute spleen weights were significantly depressed in females at all dosages of ClO_2, whereas liver weights were significantly depressed in males at 50, 100, and 200 mg/L and in females at 100 mg/L, compared with control values (Table 5). Statistical analyses of relative liver weights of the males showed the same pattern of reductions (2.59 ± 0.17 and 2.60 ± 0.27 versus 2.95 ± 0.22 g). In addition, at the 200-mg/L concentration, the mean relative kidney weight of females (0.78 ± 0.06 versus 0.67 ± 0.05 g) and the relative brain weight of males (0.43 ± 0.04 versus 0.37 ± 0.04 g) were significantly increased.

TABLE 5

Selected data on body weight, organ weight, water consumption, and daily dosage for rats exposed to chlorine dioxide in drinking water for 90 days

Parameter	Mean ± Standard Deviation				
	0 mg ClO_2/L	25 mg ClO_2/L	50 mg ClO_2/L	100 mg ClO_2/L	200 mg ClO_2/L
Males					
Initial body weight—*g*	305.1 ± 8.4	308.9 ± 11.9	305.6 ± 11.6	306.9 ± 10.1	309.8 ± 9.9
Final body weight—*g*	587.4 ± 49.7	590.2 ± 32.2	560.3 ± 49.1	558.6 ± 48.5	518.6 ± 45.8*
Weight gain—*g*	282.3 ± 47.6	281.3 ± 29.1	254.7 ± 43.8	251.7 ± 42.7	208.8 ± 38.9*
Organ weight—*g*					
Brain	2.19 ± 0.11	2.20 ± 0.11	2.11 ± 0.11	2.20 ± 0.18	2.21 ± 0.21
Testes	3.65 ± 0.28	3.58 ± 0.33	3.53 ± 0.18	3.72 ± 0.40	3.61 ± 0.43
Heart	1.71 ± 0.14	1.66 ± 0.08	1.61 ± 0.11	1.60 ± 0.19	1.60 ± 0.18
Kidneys	4.16 ± 0.40	4.40 ± 0.45	3.79 ± 0.46	4.05 ± 0.23	3.86 ± 0.37
Adrenals	0.09 ± 0.02	0.10 ± 0.04	0.10 ± 0.02	0.10 ± 0.03	0.10 ± 0.03
Liver	17.26 ± 1.46	18.02 ± 2.27	14.54 ± 1.81*	14.77 ± 1.61*	13.44 ± 1.69*
Lung	2.42 ± 0.32	2.52 ± 0.27	2.24 ± 0.21	2.26 ± 0.38	2.22 ± 0.26
Spleen	0.92 ± 0.13	0.95 ± 0.08	0.80 ± 0.19	0.88 ± 0.19	0.85 ± 0.18
Thymus	0.44 ± 0.07	0.44 ± 0.08	0.41 ± 0.07	0.38 ± 0.12	0.35 ± 0.07
Water consumption—*mL/rat/d*†	49.0 ± 5.1	44.5 ± 2.2	36.4 ± 1.6*	34.3 ± 2.4*	29.6 ± 2.3*
Calculated dose—*mg/kg/d*		1.9 ± 0.1	3.6 ± 0.7	6.2 ± 0.7	11.5 ± 1.2
Females					
Initial body weight—*g*	209.4 ± 7.0	209.2 ± 7.4	211.5 ± 7.2	218.8 ± 7.1*	208.7 ± 4.4
Final body weight—*g*	302.7 ± 15.7	298.2 ± 21.6	308.3 ± 22.5	289.6 ± 19.4	274.5 ± 28.7*
Weight gain—*g*	93.4 ± 16.3	89.0 ± 17.1	96.8 ± 20.9	70.8 ± 14.6	65.8 ± 26.2*
Organ weight—*g*					
Brain	2.05 ± 0.17	1.96 ± 0.10	1.98 ± 0.08	1.92 ± 0.08	1.95 ± 0.10
Ovaries	0.20 ± 0.05	0.19 ± 0.04	0.20 ± 0.05	0.19 ± 0.04	0.20 ± 0.03
Heart	1.03 ± 0.10	1.02 ± 0.17	0.97 ± 0.07	0.92 ± 0.05	0.96 ± 0.10
Kidneys	2.04 ± 0.18	2.13 ± 0.14	2.17 ± 0.12	2.01 ± 0.24	2.15 ± 0.20
Adrenals	0.10 ± 0.02	0.10 ± 0.01	0.10 ± 0.02	0.09 ± 0.02	0.11 ± 0.02
Liver	8.33 ± 0.66	7.85 ± 0.57	8.15 ± 1.10	7.09 ± 0.56*	7.39 ± 0.84
Lung	1.70 ± 0.37	1.48 ± 0.17	1.74 ± 0.28	1.41 ± 0.13	1.44 ± 0.10
Spleen	0.67 ± 0.12	0.53 ± 0.04*	0.57 ± 0.06*	0.54 ± 0.07*	0.53 ± 0.06*
Thymus	0.38 ± 0.10	0.32 ± 0.09	0.35 ± 0.08	0.33 ± 0.07	0.29 ± 0.07
Water consumption—*mL/rat/d*†	36.2 ± 4.7	28.8 ± 2.4*	28.0 ± 2.7*	23.5 ± 3.2*	20.4 ± 2.6*
Calculated dose—*mg/kg/d*		2.4 ± 0.3	4.6 ± 0.4	8.2 ± 1.1	14.9 ± 0.6

*Significantly different from control group; $p \leq 0.05$, Tukey's multiple-comparison procedure used
†$N = 5$; calculated on a per-cage basis
‡$N = 4$; calculated on a per-cage basis and corrected for two rats per cage

Hematological analyses indicated the females had decreased Hgb and Hct values at 25 mg/L (Table 6), and the WBC differential showed an increased percentage of lymphocytes (82.5 ± 10 versus 70.3 ± 7.6 percent) but a decreased percentage of neutrophils (11.8 ± 8.6 versus 22.9 ± 7.4 percent) at 200 mg ClO_2/L (data not shown). Clinical serum chemistry showed several differences in both sexes compared with the controls (Table 6). Females had significantly decreased Creat levels at 50 and 200 mg/L but exhibited significantly increased PO_4 levels after drinking 100 mg ClO_2/L. In males, Creat was significantly increased at 100 and 200 mg/L, and PO_4 was increased at 100 mg/L but not at the highest concentration. Decreased levels of AST and LDH at 100 and 200 mg/L and of ALT at 25 and 50 mg/L were also noted in males.

Histopathological examination identified the nasal cavity as the target tissue (Table 7). The histopathologic changes seen in both sexes at all ClO_2 treatment levels were characterized by various types of inflammation ranging from acute to chronic or active, goblet cell and epithelial cell hyperplasia and squamous metaplasia. Incidences of goblet cell hyperplasia and subacute inflammation of the nasal turbinates in males were significantly increased over controls at all dosage levels. The majority of the changes were present in the most anterior section of the nasal cavity, with the nasal septum the most affected area.

Discussion

This article describes detailed, comparative toxicity studies of three disinfectants: chlorine, monochloramine, and chlorine dioxide. These chemicals were administered via drinking water to adult Sprague-Dawley rats of both sexes for 90 days. Detailed hematological, chemical, and histopathological examinations were conducted. There were clear differences in the toxicological effects of the disinfectants. Chlorine exposures could not be associated with a dosage-related toxicological effect in either sex, even at the highest concentration of 250 mg/L. Conversely, NH_2Cl induced some significant organ weight changes at the highest concentration (200 mg/L) in both sexes. Chlorine dioxide was, by far, the most toxic agent, producing body and organ weight losses at the higher concentrations, as well as significant histopathological effects, even at the lowest concentration (25 mg/L).

Chlorine. In aqueous media, chlorine reacts with water, forming hypochlorous acid (HOCl) in addition to its reduction product, chloride ion. Hypochlorous acid is a weak acid and dissociates to hypochlorite ion (OCl^-) as pH increases.[22] The ingestion of aqueous chlorine for 90 days in this study did not produce toxicological effects. The body and organ weights of the treated animals were similar to those of the controls, and hematological and clinical chemistry evaluations indicated no dosage-related effects. Further, no target organs could be identified from the histopathological examination.

The absence of toxicological effects seen in this study corroborates previous, although less detailed, studies of the administration of chlorine from aqueous medium (as hypochlorous acid or sodium hypochlorite) to experimental animals. For example, Hasegawa et al report that the administration of 500–2,000 mg/L sodium hypochlorite (240–960 mg/L available chlorine) to Fischer 344 rats (both sexes) for 104 weeks, and 500–4,000 mg/L for 90 days, resulted in reductions in body-weight gain as the only indication of toxicity.[23] No significant hematological or histopathological effects (relative to control animals drinking distilled water) were observed; however, based on the studies reported in this article, it seems remarkable that the animals could be induced to drink these concentrations. Similarly, Cunningham observed no evidence of toxicity in male rats drinking water containing hypochlorite as 0, 20,

TABLE 6

Selected hematological and clinical chemistry values for rats treated with chlorine dioxide in drinking water for 90 days

Parameter	Mean ± Standard Deviation				
	0 mg ClO_2/L	25 mg ClO_2/L	50 mg ClO_2/L	100 mg ClO_2/L	200 mg ClO_2/L
Males					
RBC × 10^6	8.10 ± 0.39	8.14 ± 0.29	8.06 ± 0.41	7.99 ± 0.33	8.26 ± 0.42
WBC × 10^3	6.55 ± 1.19	6.76 ± 1.26	6.75 ± 1.85	6.56 ± 1.80	6.29 ± 1.87
Hgb—*g/dL*	14.94 ± 0.79	15.12 ± 0.56	15.06 ± 0.86	15.10 ± 0.74	15.31 ± 0.55
Hct—*percent*	43.88 ± 2.52	44.36 ± 2.01	44.23 ± 2.71	44.06 ± 2.39	45.25 ± 2.81
MCV—u^3	54.60 ± 1.43	54.80 ± 1.55	55.10 ± 1.91	55.40 ± 1.71	55.30 ± 1.64
Glu—*mg/dL*	153.50 ± 40.89	141.50 ± 11.04	151.60 ± 31.33	173.10 ± 29.67	176.60 ± 35.33
BUN—*mg/dL*	19.34 ± 2.22	18.05 ± 2.14	18.94 ± 7.96	23.97 ± 3.69	19.86 ± 1.62
Creat—*mg/dL*	0.60 ± 0.13	0.59 ± 0.07	0.63 ± 0.07	0.79 ± 0.10*	0.72 ± 0.09*
PO_4—*mg/dL*	7.08 ± 0.93	7.65 ± 1.02	7.72 ± 0.90	8.48 ± 0.63*	8.02 ± 1.21
AST—*U/L**	128.30 ± 37.51	92.10 ± 21.90	87.10 ± 25.53	86.00 ± 23.16*	80.90 ± 20.32*
ALT·P—*U/L*	49.60 ± 13.02	36.80 ± 7.32*	34.00 ± 5.21*	40.40 ± 6.35	39.90 ± 6.52
Chol—*mg/dL*	61.30 ± 17.03	62.20 ± 12.82	67.50 ± 13.57	57.70 ± 13.06	63.10 ± 14.04
LDH—*U/L**	801.4 ± 476.8	53.36 ± 342.9	479.5 ± 161.1	313.1 ± 304.6*	301.6 ± 227.6*
Ca—*mg/dL**	10.45 ± 0.70	9.95 ± 0.36	10.15 ± 0.37	10.04 ± 0.36	10.16 ± 0.39
Females					
RBC × 10^6	7.48 ± 0.49	6.96 ± 0.25	7.35 ± 0.33	7.39 ± 0.21	7.34 ± 0.63
WBC × 10^3	3.94 ± 1.26	2.96 ± 0.70	3.92 ± 1.21	4.08 ± 1.12	4.97 ± 1.96
Hgb—*g/dL*	14.83 ± 0.67	13.99 ± 0.45*	14.78 ± 0.41	14.54 ± 0.40	14.27 ± 0.77
Hct—*percent*	42.50 ± 2.93	39.10 ± 1.67*	41.56 ± 1.98	41.71 ± 1.19	40.89 ± 3.20
MCV—u^3	57.00 ± 1.56	56.50 ± 1.27	56.80 ± 1.48	56.60 ± 1.43	56.10 ± 1.37
Glu—*mg/dL*	121.10 ± 23.29	124.20 ± 15.43	120.90 ± 16.27	127.10 ± 11.32	144.20 ± 23.47
BUN—*mg/dL*	19.61 ± 3.44	15.39 ± 2.41	20.32 ± 7.47	17.45 ± 3.00	18.10 ± 2.81
Creat—*mg/dL*	0.71 ± 0.10	0.63 ± 0.11	0.59 ± 0.03*	0.66 ± 0.11	0.59 ± 0.07*
PO_4—*mg/dL*	3.96 ± 1.20	6.79 ± 0.99	7.17 ± 0.99	7.81 ± 0.68*	6.64 ± 0.93
AST—*U/L*	86.90 ± 20.78	86.80 ± 20.23	108.40 ± 19.74	73.50 ± 20.49	75.80 ± 14.82
ALT—*U/L*	40.70 ± 8.37	39.90 ± 7.72	41.60 ± 7.59	35.50 ± 6.42	35.10 ± 8.25
Chol—*mg/dL*	67.50 ± 14.30	70.20 ± 18.13	83.00 ± 16.95	67.30 ± 12.47	71.40 ± 19.25
LDH—*U/L*	556.6 ± 431.3	543.7 ± 417.5	629.2 ± 307.7	275.7 ± 220.9	342.4 ± 201.8
Ca—*mg/dL*	10.88 ± 0.46	10.50 ± 1.45	10.83 ± 0.50	10.58 ± 0.56	10.53 ± 0.68

*Significantly different from control group; $p \leq 0.05$, multiple-comparison procedure based on Tukey's studentized range test unless otherwise indicated

40, or 80 mg/L available chlorine (0, 4.1, 8.1, or 15.7 mg/kg/d) for six weeks or in guinea pigs that consumed 50 mg/L available chlorine (13.4 mg/kg/d) for five weeks.[24]

The administration of 500–1,000 mg/L sodium hypochlorite to $B_6C_3F_1$ mice of both sexes for 106 weeks resulted in no evidence of treatment-related cancers or other toxicological effects.[25] Finally, studies conducted on white mice showed no toxicological effects associated with the consumption of 100 or 200 mg/L free available chlorine (pH 5.9–6.2) during 50 days of treatment.[26]

On the other hand, some investigators have observed transient, although reversible, decreases in blood glutathione and hypothalamic norepinephrine levels, as well as morphological and biochemical changes, in the liver of rats gavaged for one to 14 days with 250 mg/kg/d HOCl.[27-29] Further, there is evidence that the administration of drinking water containing 2–15 mg/L chlorine (as either HOCl or OCl^-), given in conjunction with a hypocalcemic diet,[12] may produce increased serum cholesterol, as well as myocardial hypertrophy and arteriosclerosis in rabbits and pigeons.[30] However, these latter studies are in need of verification.[31]

In summary, based on this study, the 250-mg/L concentration (24.9 and 16.7 mg/kg/d for females and males, respectively) for 90 days is, therefore, considered the no observable adverse effect level (NOAEL). These values are consistent with the no observable effect level (NOEL) found in Blabaum and Nichols' study of mice at 200 mg chlorine/L (approximately 25 mg/kg/d, assuming 0.02 kg body weight and 0.0025 L/d water consumption).[26]

Monochloramine. The most significant toxicological effects observed in this 90-day toxicity study with monochloramine were the reductions in body-weight gain and organ weights. In males the significant reduction in body-weight gain was observed at doses of 50 mg/L and higher; in females the reduced weight gain was significant only at the higbest dosage. For both males and females in the 200-mg/L dose group, the average weight gain was approximately 51 percent of that of the controls.

At the highest dosage level, reductions in organ weights (absolute, relative, or both) were observed for both males and females, with liver and spleen weights decreased in both sexes. Although these weight reductions appeared to be dosage-related for the males, subsequent histopathological examination did not reveal any target organs or any treatment-related changes. On the other hand, in an earlier study, similar toxicological effects (e.g., decreased body-weight gain and liver effects) were reported for rats and mice administered high (200–400 mg/L) concentrations of NH_2Cl in drinking water for 90 days.[15]

In addition, in the males, the reduced RBC count (100 and 200 mg/L) and the dosage-related decrease in serum calcium levels were significant. However, these clinical effects were considered not biologically significant, not dosage-related, or within the normal range for rats of this age and strain.[32]

When the actual dosage of monochloramine was computed, using individual body weight and water consumption, the males were exposed to about 18–30 percent less chemical, on a milligram-per-kilogram-per-day basis, than females administered an equivalent concentration.

Human patients undergoing long-term dialysis developed hemolytic anemia when water containing NH_2Cl was inadvertently used in the treatment.[9] However, no such hematological effects of NH_2Cl have been sbown in drinking-water exposures with several laboratory species, including mice,[10] rats,[11] pigeons,[12] and monkeys[13] at concentrations up to 200, 100, 15, and 100 mg/L, respectively. Revis et al[12] observed increased levels of plasma cholesterol and an increase in mean aortic plaque size in

TABLE 7

*Results of histopathological examination of the nasal turbinates of rats treated with chlorine dioxide for 90 days**

Lesion	Number of Animals				
	0 mg ClO_2/L	25 mg ClO_2/L	50 mg ClO_2/L	100 mg ClO_2/L	200 mg ClO_2/L
Males					
Hyperplasia, goblet cell	0	6†	5‡	9§	10§
Metaplasia, squamous	0	4	2	0	1
Inflammation, subacute	0	7†	7†	9§	8§
Females					
Hyperplasia, goblet cell	2	6	6	7‡	7‡
Metaplasia, squamous	0	3	1	3	0
Inflammation, subacute	0	2	3	10§	8§

*Number of animals showing the indicated lesion at necropsy; each group contained 10 rats.
†$p \leq 0.01$, Fisher's Exact test
‡$p \leq 0.05$, Fisher's Exact test
§$p \leq 0.001$, Fisher's Exact test

the pigeon following a 30-day exposure to 15 mg/L in drinking water. However, a reanalysis of the Revis et al study suggested that these experiments be repeated for confirmation.[31]

Thus, for this study, the 100-mg/L concentration equivalent to 7.71 and 5.79 mg/kg/d monochloramine for female and male rats, respectively, can be considered a NOAEL. This compares well with the NOEL of 8.3 mg/kg/d suggested by others for the Fischer 344 rat and the $B_6C_3F_1$ mouse[15] and with the NOEL of 10.0 mg/kg/d reported by Bercz and co-workers for the African Green monkey.[13] A matched watering and feeding study would be useful for distinguishing between systemic toxic effects and weight loss from taste aversion and would more clearly identify the NOAEL. Likewise, from this study, 200 mg/L might be considered a lowest observable adverse effect level (LOAEL) on the basis of the observed alterations in absolute and relative organ weights.

Chlorine dioxide. The three highest dosages of ClO_2 used in this 90-day study caused dosage-related decreases in water consumption (both sexes) and in food consumption at the highest dosage for the males. Decreased water consumption is most likely caused by a taste aversion. However, decreased food consumption may be indicative of a disinfectant-induced, systemic toxicity. A matched watering and feeding study could discriminate between these effects. Body weight and weight gain were significantly reduced (>10 and 26 percent, respectively) in both sexes at 200 mg ClO_2/L, and the absolute and relative liver weights of both sexes and spleen weights in females showed a dosage-related reduction. Although the hematological and clinical chemistry determinations revealed no dosage-related effects, the changes in several enzymes (AST, LDH) in males suggest liver toxicity.

Histopathologic examinations identified the nasal turbinates of both sexes as the only target tissue. Goblet cell hyperplasia was significantly increased at 100 and 200 mg/L in females and at all ClO_2 treatment levels in males. A subacute inflammation of the nasal cavity was significantly increased in males at 25 mg/L and in both sexes at higher concentrations of ClO_2.

Toxicological effects of ClO_2 to the hemopoietic system have been well documented.[6] The ability of ClO_2 to oxidize hemoglobin, producing methemoglobinemia and accompanying hemolytic anemia, has received considerable attention from Abdel-Rahman and co-workers.[33-35] In a series of studies, these workers supplied mice and rats with drinking water containing 1-1,000 mg ClO_2/L for periods of up to one year. These experiments resulted in doses of approximately 0.1-10 mg/kg/d for rats and 0.18-18 mg/kg/d for mice (1-100 mg/L), doses comparahle to those used in this study. The most consistent toxicologic finding in these studies was an increase in RBC catalase activity in rats drinking 100 mg ClO_2/L and in mice consuming 10 and 100 mg ClO_2/L. Similarly, increased levels of deformed RBCs (echinocytes) were reported in rats after four months of exposure, but a resistance of RBCs to hemolysis (decreased osmotic fragility) developed in rats exposed to 1-10 mg ClO_2/L for nine months.

In contrast, Moore and Calabrese[36] treated mice with 18 mg/kg/d of ClO_2 (drinking water exposure) for 30 days and observed no effects on several hemotological parameters, including glucose-6-phosphate dehydrogenase activity, RBCs, hematocrit, WBCs, mean corpuscular volume, mean corpuscular hemoglobin, reticulocyte levels, and RBC osmotic fragility. Similarly, Bercz and co-workers offered African Green monkeys drinking water containing 0, 30, 100, or 200 mg ClO_2/L for six weeks (equivalent to 3.5, 9.5, and 11.0 mg/kg/d, respectively) and found that water consumption was inversely related to dosage. Monkeys in the 200-mg/L group displayed erythema and ulceration of the oral mucosa.[13] Ingestion of ClO_2, however, did not produce significant changes in any of the hematological or serum biochemical parameters, including red cell count and methemoglobin levels, that were measured in this species. Studies by Revis et al[12] have indicated that treatment of male white carneau pigeons with drinking water containing 15 mg ClO_2/L in conjunction with a high-fat diet resulted in enlargement of the aortic plaques.

One report of a chronic study, conducted 41 years ago, concluded that the administration of 100 mg ClO_2/L in drinking water (equivalent to 12.5 and 13.4 mg/kg/d in males and females, respectively) reduced the mean life span of rats from 85 to 58 weeks but resulted in no observable, treatment-related histopathological effects.[37]

Histopathologic changes of the type seen in this study for ClO_2 have been reported in rats inhaling other acrid compounds such as chlorine and hydrogen chloride. Rats exposed to 9 ppm chlorine gas for 6 h/d, 5 days/week for six weeks developed inflammation of the upper and lower respiratory tract with necrotic lesions appearing in the nasal turbinates.[38] Similarly, $B_6C_3F_1$ mice exposed to hydrogen chloride vapors at 50 ppm developed rhinitis in the anterior portion of the nasal cavity and eosinophilic globules in the epithelial lining of the nasal tissue.[39]

Studies relating these histopathologic findings to the oral route of exposure are not available. But it seems likely that the nasal lesions found in this study were due to inhalation of ClO_2 vapors at the sipper tube or from off-gassing of the vapors after drinking. Until further research quantifies the level of inhalation exposure ClO_2 associated with drinking water containing the chemical, the interpretation of this study would indicate an oral LOAEL of 25 mg/L (~1.9 mg/kg/d).

Conclusion

These studies establish that the subchronic toxicity of the three disinfectants in rats is chlorine dioxide >> monochloramine > chlorine, when administered in drinking water. Two points must be kept in mind: (1) the actual concentration of disinfectant required to disinfect a water supply depends on its efficacy as a biocide, thus allowing a powerful agent like chlorine dioxide to be used at a lower concentration than some less toxic, although less effective, agent; and (2) proper consideration must also be given to the toxicity of the by-products that inevitably result when powerful oxidants like those considered here react with exogenous natural products (e.g., humic acids) present in the water at the time of disinfection. Thus,

the relative toxicity directly attributed to these three agents is not the only factor that will influence the selection of a drinking water disinfectant.

Acknowledgment

The authors thank Peter Bercz of USEPA's Health Effects Research Laboratory for doing the hematology and serum chemistries for these studies and Kay Carr for preparing the manuscript. This article has been reviewed by the Health Effects Research Laboratory and has been approved for publication. Mention of trade names or commercial products does not constitute an endorsement or recommendation for use.

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